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Dietary Boron: An Overview of the Evidence for Its Role in Immune Function

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This review summarizes the evidence for boron essentiality across the biological spectrum with special focus on biochemical pathways and biomolecules relevant to immune function. Boron is an essential trace element for at least some organisms in each of the phylogenetic kingdoms Eubacteria, Stramenopila (brown algae and diatoms), Viridiplantae (green algae and familiar green plants), Fungi, and Animalia. Discovery of several of the currently recognized boron-containing biomolecules was achieved because the bound boron formed four coordinate covalent bonds with the ligand, creating a thermodynamically stable complex that is almost undissociable in water. Boron is a constitutive element in three antibiotics and a quorum-sensing signal in bacteria. It enhances Fc receptor expression and interleukin-6 production in cultured mammalian macrophages. Boron binds tightly to the diadenosine polyphosphates and inhibits the in vitro activities of various serine protease and oxidoreductase enzymes. Physiological amounts of dietary boron decrease skinfold thickness after antigen injection in gilts and elevated circulating natural killer cells after adjuvant injection in rats. It is predicted that several boron biomolecules waiting discovery are signaling molecules that interact with the cell surface and are probably composed of two mirror or near-mirror halves stabilized by a single boron atom to form a large circular biomolecule. J. Trace Elem. Exp. Med. 16:291–306, 2003. Published 2003 Wiley-Liss, Inc.†

Key words: boron; immune function; boron essentiality; natural killer cells; boroesters

BORON CHEMISTRY

Chemical Characteristics

The biomolecules known to contain boron are either directly involved in immune defense mechanisms or affect components of the immune system of a particular organism. The unusual nature of boron chemistry is summarized here to serve as a guide to the discovery of other boron biomolecules involved in immune function. Although many synthetic boron compounds are made in the laboratory, boron does not occur free nor bind directly to any element other than oxygen in geological systems except for trivial exceptions [1]. Only those organic compounds that contain B-O or B-N bonds (the organoboron compounds) are in important in biological systems during normal physiological conditions. Exper-

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imental evidence to date suggests that these organoboron complexes are the result of interaction with either OH or amine groups as described below. Organoboron compounds or complexes are present in species across the biological spectrum.

Boric acid (proper chemical name: orthoboric acid), $B(OH)_3$, is the most probable form of boron after ingestion and subsequent hydrolysis [1], and therefore available for interaction with biomolecules. Notably, boric acid is not a proton donor, but rather accepts a hydroxyl ion (a Lewis acid) and leaves an excess of protons to form the tetrahedral anion $B(OH)_4^-$ (Reaction 1) [2].

$$B(OH)_3 + 2H_2O \Leftrightarrow H_3O^+ + B(OH)_4^- \; pKa = 9.25(25^oC) \quad (1)$$

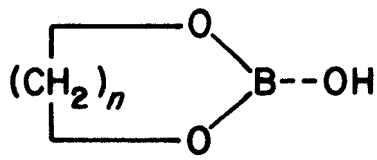
Thus, at typical physiological boron concentrations (6.0×10^{-7} to $\sim 9.0 \times 10^{-3}$ mol/L) in plants, animals, or humans, inorganic boron is essentially present only as the monomeric species boric acid $B(OH)_3$ and as borate $B(OH)_4^-$ [3]. With a p*K*a in solution of 9.2, boron is predominantly in the uncharged, planar, trigonal boric acid form (H_3BO_3) at physiological pH. However, when boron forms covalent bonds with biological ligands, its p*K*a is reduced to ~6 [4], and the majority of the boron in ligand complexes are in the negatively charged borate ($H_4BO_4^-$) form while occupying the binding site [2,3].

Boroesters

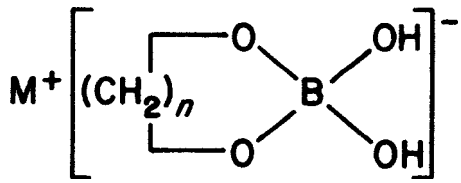
Boron oxo compounds can react with many biomolecules that contain one or more hydroxy groups or similar suitable molecular structures to form boroesters, one of the only two known classes of biologically relevant boron species. Several types of boron esters exist. Boric acid reacts with suitable dihydroxy compounds to form corresponding boric acid monoesters (''partial'' esterification, e.g., Structure 1) that retain the trigonal-planar configuration and no charge.

In turn, a boric acid monoester can form a complex with a ligand containing a suitable hydroxyl to create a borate monoester (''partial'' esterification; monocyclic, e.g., Structure 2) but with a tetrahedral configuration and a negative charge. A compound of similar configuration and charge is also formed when borate complexes with a suitable dihydroxy compound. The two types of boromonoesters can react with another suitable dihydroxy compound to give a corresponding spiro-cyclic borodiester (''complete'' esterification) that is a chelate complex with a tetrahedral configuration and negative charge (Structure 3) [5]. A partially esterified tridentate cleisto complex (Structure 4) may be formed when a ligand contains three suitably *cis*-oriented hydroxyl groups [6].

Not all diol ligands can react with boric acid or borate because the resultant boroester must contain O-B-O angles that do not exceed the limits of tolerable strain on the bond. Typically, ligands that contain adjacent *cis* hydroxyl groups are most likely to react with B oxo compounds to form a boroester, and the reactivity of boric acid with the ligand generally increases in proportion to the number of these *cisoid* groups [7]. The relevant *cis*-diol conformations for boron complexation are present in several biologically important sugars, their derivatives (sugar alcohols, -onic, and -uronic acids), and some polymers. A boroester is sometimes formed without the presence of a *cisoid*-diol group because of stereochemistry that limits bond strain [7].



Structure 1.



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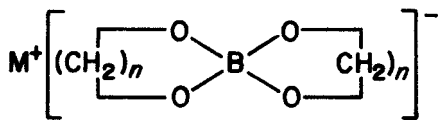

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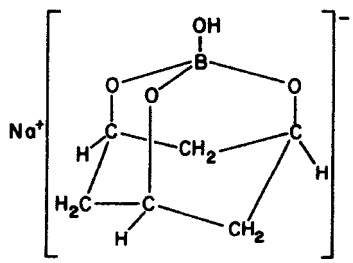
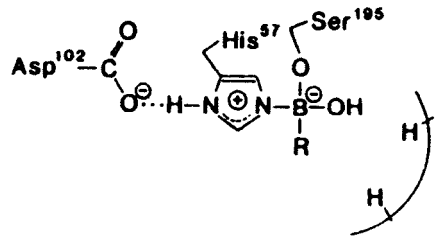


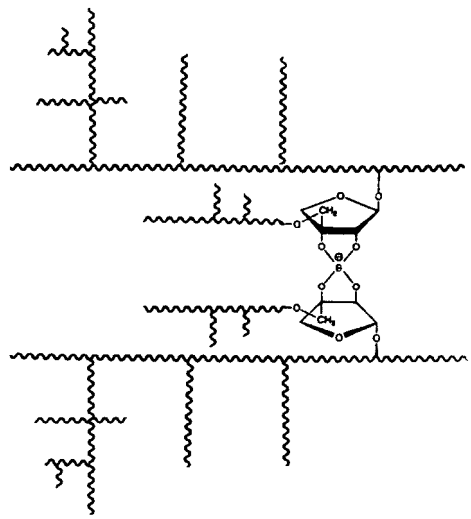
Structure 4.

Also, hydroxyl groups arising from lactol formation (in glucose, alpha-D-form only), hydration of COOH groups (in alpha- hydroxy, e.g., lactic acid; and aromatic o-hydroxy acids, e.g., salicylic acid), are reactive with $B(OH)_3$ [7]. As discussed below, the form of glucose in biological systems (nearly all in the pyranose, not furanose, form and therefore unavailable for complexation with boron) was probably an early driving force in the evolution of fungi, plants, and animals.

Discovery of functional biological boromonoesters (e.g., Structure 1) is especially challenging because the esterification reaction that produces this type of ester is easily reversible [8,9]. However, the discovery of several of the currently recognized boron-dependent biomolecules was achieved because the bound boron formed four coordinate covalent bonds with the ligand, creating a thermodynamically stable complex (Structure 3) that is almost undissociable in water [10,11].



Structure 5.

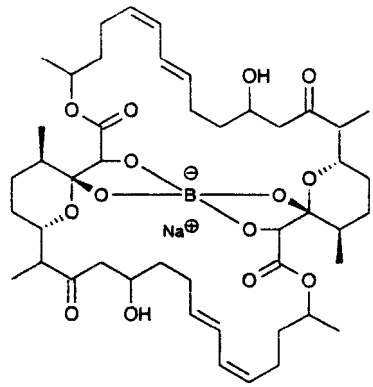


Structure 6.

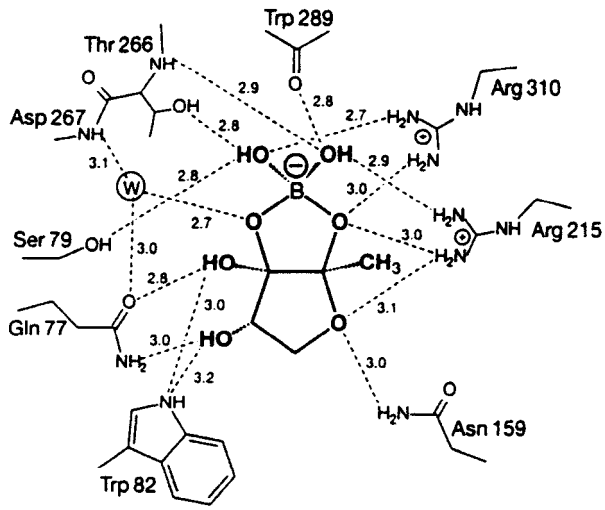
At the molecular level, boron influences the activities of at least 26 enzymes (most often in an inhibitory manner) examined in various animal, plant, cell-culture, and refined chemical reaction systems. It appears to do this by acting on the enzyme directly, by binding to cofactors (e.g., NAD) or substrates, as well as other presently unclear mechanisms [12–14]. Oxidoreductase enzymes that require pyridine (e.g., NAD^+ or NADP) or flavin (e.g., FAD) nucleotides (EC 1.1.1, 1.1.3, 1.2.1, 1.3.5, 1.6.2) are competitively inhibited by borate or its derivatives as boron competes for the NAD or flavin co-factor. Reversible enzymatic inhibition as an essential role for an element is unusual. However, there is irrefutable evidence that boron serves to inhibit or dampen several metabolic pathways in higher plants.

Nitrogen–Boron Compounds

Organoboron compounds also include B-N compounds because B-N is isoelectronic with C-C [2]. A variety of biological complexes important in the inflammatory process are formed when nitrogen acts as an electron-pair donor to



Structure 7.

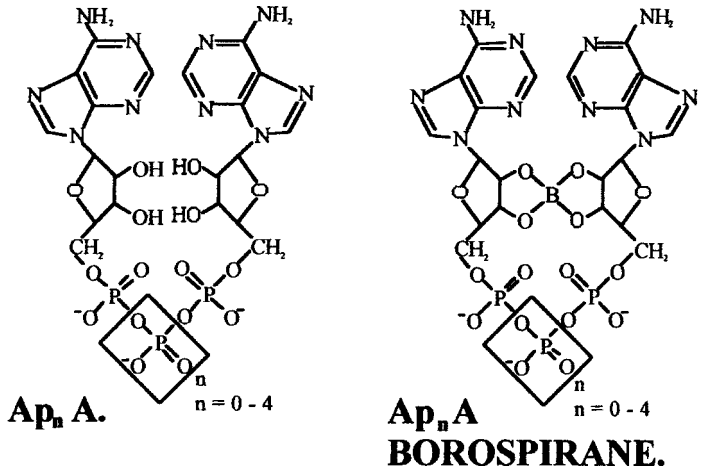


Structure 8.

fill the vacant boron p_z orbital. For example, experimental evidence [15] suggests that the mechanism for inhibition of a specific sub-subclass of enzymes (the serine proteases) by boron involves formation of a covalent bond between boron and a specific nitrogen at the active site of these enzymes. By way of further example, the $N^{\epsilon 2}$ of histidine-57 of α-lytic protease and the boron atom of a peptide boronic acid interact to form a covalent bond and give rise to a reversible complex (Structure 5). Many serine proteases are involved in the inflammatory process and their relation to boron is discussed below.

ESTABLISHED NONIMMUNOLOGICAL ROLES FOR BORON

Examination of a role for boron in immune function is aided by an appreciation of the beneficial physiologic effects of boron across the biological spectrum.



Structure 9.

Prokaryotes (Organisms Without Limiting Membrane Around Nuclear Material)

Within the kingdom Eubacteria, boron is important for organisms distributed over at least three separate phyla (Actinobacteria, Proteobacteria, and Cyanobacteria). The first boron biomolecule to be discovered is an antibiotic produced by *Streptomyces antibioticus*, bacteria that are assigned to the phylum Actinobacteria. This and other boron-containing bacterial products from other species within Actinobacteria and Proteobacteria are directly related to the immunological defense of the organism and therefore discussed more fully below.

In the phylum Cyanobacteria (blue–green algae), boron is required for several dinitrogen-fixing bacteria with heterocysts (*Nodularia* sp., *Chlorogloeopsis* sp., Nostoc sp., and *Nostoc* sp. *PCC 7119*) but apparently not for the nonheterocystous, dinitrogen-fixing forms or for the nondinitrogen fixing forms of Cyanobacteria [16]. Heterocysts are specialized cells on some filamentous cyanobacteria and engage in nitrogen fixation. It was suggested that boron stabilizes the glycolipid inner layer of the heterocysts by interacting with their hydroxyl groups [16]. Boron essentiality for the heterocystous Cyanobacteria, predominant organisms during the Middle Pre-Cambrian Period, indicates that boron was an essential element during the early evolution of life [16].

Eukaryotes (Organisms With Limiting Membrane Around Nuclear Material)

Kingdom Stramenopila. The kingdom Stramenopila is a newly recognized split from the older diverse kingdom Protista that included all eukaryotic organisms that are not animals, true fungi, or green plants. Stramenopila is an important group of organisms some of which have the largest linear dimensions known (brown algae) or are ecologically very important (diatoms). Boron is required by species of brown algae and diatoms. For example, embryos of *Fucus*

edentatus, a species of brown algae, die within four weeks in a boron-free medium [17]. Cells of *Cylindrotheca fusiformis*, a marine pennate diatom, are unable to divide in the complete absence of boron [18]. Likewise, 11 other marine pennate diatoms, 4 marine centric diatoms, and 8 freshwater diatom species have an established boron requirement [19].

Kingdom Fungi. Fungi share more genetic and protein homologies with mammals than they do with green plants. This is of special interest in the field of boron nutrition because of findings in 1999 [20] that boron stimulated growth in the fungus *Saccharomyces cerevisiae* (Brewer's yeast) during both the log growth phase and stationary growth phase. This finding supplanted those published in 1968 [21] to the contrary. Boron deficiency also greatly reduces growth of the fungal species *Dothiorella* sp. [22]. Because fungal species have a demonstrated physiological response to supplemental boron and are a useful model for examining basic biomolecular mechanisms, their further study will aid in the search for the precise biochemical role of boron in humans and other animals.

Kingdom Viridiplantae (Green plants). The two major lineages of green plants are Chlorophyta (green algae) and Streptophytes (familiar green plants found mostly on land plus some organisms traditionally considered green algae). The use of sucrose instead of carbohydrates (which contain cis-diols that could react with boric acid) as the major storage and transport sugar is thought to be an important evolutionary change that "freed up" boron for other uses and allowed evolution of the green algae. That is, the high affinity of boric acid for adjacent and *cis*-hydroxyl groups present on biomolecules may have exerted considerable evolutionary pressure to select for carbohydrate energy sources with low percentages of the furanose forms [23]. However, there may have been selection for two natural sugars, apiose and ribose, because their physiological derivatives have a strongly-borate-complexing furanose configuration [23] and they serve as components of important structural or enzyme-related biomolecules. As described more fully below, apiose moieties are found in the cells of many higher plants. Even in a marine green alga (*Ulva lactuca*), boric acid (with Ca^{2+}) stiffens the structure of its sulfated rhamnose-containing polysaccharides [24], a finding that led to the hypothesis that *Ulva* regulates the stiffness of its polysaccharide gel by adding or removing sulphate groups [25].

Boron is recognized as essential for all species of vascular plants (Tracheophyta) but, surprisingly, the primary function is still unknown [13]. Recent evidence suggests that the predominant place of boron function in plants is in the primary cell walls where it cross-links the pectic polysaccharide rhamnogalacturonan-II (RG-II). The RG-IIs are small, structurally complex polysaccharides that represent an extreme example of the evolutionary conservation of wall polysaccharide structure [26]. They comprise one region in a long chain of polysaccharides that forms pectin in primary plant cells. The RG-IIs have side chain sugar residues that are characterized by rare sugars including apiose. Apiose can cyclize only as a furanose such that, in aqueous solution, the predominate (54%) free form is β-D-erythrofuranose, a form that represents the optimal configuration for complexation with borate [23]. Thus, an atom of boron crosslinks two RG-II monomers at the site of the apiose residues to form a

borodiester (Structure 6) [27] and multiple crosslinks form a supramolecular network. As described below, RG-II dimers influence a component of mammalian immune function.

In plants, a serious outcome of boron deficiency is the accumulation of starch in chloroplasts and acceleration of the pentose phosphate cycle [28]. Although the mechanism responsible for this phenomenon is not fully understood, it seems clear that boron inhibits the activities of specific enzymes involved in starch metabolism or within the pentose cycle. The pentose cycle is active in mammalian systems, and is the basis for a proposed mechanism for the action of boron in neutrophil respiratory burst as described below [29].

Kingdom Animalia. Boron is essential for embryological development in at least two separate vertebrate phylogenetic classes. In the South African clawed frog *Xenopus laevis*, boron deprivation disrupts embryonic development in a number of ways including a high proportion of necrotic eggs and abnormal development of the gut [30]. In mated zebrafish (*Danio rerio*), the early cleavage stage of development is sensitive to boron deficiency and repletion of low-boron embryos during the first hour after fertilization rescued them from death [31].

Boron has demonstrated beneficial effects in at least three other animal models of human nutrition with many of the effects related to bone metabolism. For example, boron supplementation of a low-boron diet reduced gross bone abnormalities in the vitamin D-deficient chick [32,33]. In vitamin D-deficient rats fed a low-boron diet, supplemental dietary boron enhanced the apparent absorption and retention of calcium and phosphorus and increased femur magnesium concentrations [34]. In male pigs, bone lipid was lower and bending moment higher when boron was supplemented to a boron-low diet [35].

ESTABLISHED IMMUNOLOGICAL ROLES FOR BORON

There is evidence from several laboratories that dietary boron has a role in immune function in a variety of organisms as summarized below.

Boron and Bacterial Products

Boromycin and similar antibiotics. The first natural biomolecule found to contain boron was boromycin, an antibiotic from a strain of *Streptomyces antibioticus* (phylum Actinobacteria) first obtained from an African soil sample [36]. Boromycin from *Streptomyces* sp. strain MA 4423 is active against Gram-positive bacteria and certain fungi and protozoae but is inactive against Gram-negative bacteria [37]. Its interaction with the cytoplasmic membrane that results in the breakdown of the permeability barrier for potassium ions [38]. Boromycin from *Streptomyces* sp. strain A-3376 was recently found to be a potent anti-human immunonodeficiency virus (HIV) antibiotic [39]. It strongly inhibits the replication of the clinically isolated HIV-1 strain and apparently blocks release of infectious HIV particles from cells chronically infected with HIV-1 by unknown mechanisms.

Tartrolon B (Structure 7) is another boron-containing antibiotic, closely related in structure to boromycin [40]. It is produced by the myxobacterium *Sor-*

angium cellulosum strain So ce678 (phylum Proteobacteria) isolated from a soil sample collected near Braunschweig, Germany. Tartrolon B acts against Gram−positive bacteria and, notably, strongly inhibits growth of mammalian cells (mouse fibroblasts) in culture [41]. A third similar boron-containing antibiotic, aplasmomycin, is excreted by a marine isolate belonging to *Streptomyces griseus* (phylum Actinobacteria) [42]. It has inhibitory activity against Gram-positive bacteria in vitro and plasmodium in vivo when administered orally to mice infected with *Plasmodium berghei* [42].

The greater stability of borodiesters compared with boromonoesters probably explains why the boron-containing antibiotics were isolated. These biomolecules are ionophoric macrolides and have four inward directed oxygen atoms (two *cis*-hydroxyl groups) that provide an ideal geometry for accommodation of the boron atom and thus formation of a stable borodiester [43]. Even so, boron can be removed from at least one of these antibiotics (aplasmomycin) by mild acid hydrolysis by using citric acid buffer solution (pH 3). This causes slight conformational changes in the molecule and formation of a new compound (desboroaplasmomycin) that does not have the ability of aplasmomycin to transport K^+ across a membrane [44]. Boron can be easily reinserted into desboroaplasmomycin by treatment with boric acid at pH 6 and 8 [45]. The overall shape of the boromycin anion is roughly spherical with a lipophilic surface and a cleft lined with oxygen atoms [46]. Assuming evolutionary conservation of early biomolecules, the chemical structures of the boron-containing antibiotics most likely serve as guideposts towards the future discovery of boron-containing biomolecules in higher organisms.

Quorum sensing signal. Recently, there was the discovery of a boron-containing biomolecule produced by a bacterium that is not an antibiotic [47] but rather a cell-to-cell communication signal. Communication between bacteria is accomplished through the exchange of extracellular signaling molecules called autoinducers (AI). This process, termed "quorum sensing," allows bacterial populations to coordinate gene expression for community cooperative processes such as antibiotic production and virulence factor expression. AI-2 is produced by a large number of bacterial species and contains one boron atom per molecule. Not surprisingly, it is derived from a ribose moiety, S-ribosylhomocysteine. The gliding bioluminescent marine bacterium, *Vibrio harveyi* (phylum Proteobacteria), produces and also binds AI-2. In *V. harveyi*, the primary receptor and sensor for AI-2 is the protein LuxP that consists of two similar domains connected by a three-stranded hinge. The AI-2 ligand binds in the deep cleft between the two domains to form a furanosyl borate diester complex (Structure 8) [47].

Boron and Animal Cell Signaling

Macrophage Fc receptor. The Fc receptor internalizes antigen-antibodies complexes and thus is necessary for efficient processing of antigens into peptides presented by major histocompatibility complex class II molecules [48]. Expression of the Fc receptor on mouse macrophages in culture was enhanced in the presence of a specific rhamnogalacturonan II (GL-4IIb2) that was isolated from

the leaves of *Panax ginseng* C.A. Meyer [49]. The concentration of boron in this rhamnogalacturonan II is relatively high (0.09%). The roots of this plant are the source of a well-known Chinese crude drug used widely for the treatment of gastrointestinal disorders and as an erythropoietic.

Interleukin-6 (IL-6). IL-6 is a systemic "proinflammatory" and, as such, an important regulator of the immune system by inducing several processes including differentiation of B-cells into high level antibody-producing cells, thymocytes into cytotoxic T-cells, and stimulation of NK-cell activity, acute phase reactant proteins, and prostaglandins [50]. Of the RG-IIs isolated from *Panax ginseng* leaves, both GL-4IIb2 and GL-RIII were relatively potent enhancers of IL-6 production in macrophages harvested from mice [51]. Notably, in the case of GL-RIII, dissociation of the RG-II dimer that contained borate diester to the monomer significantly decreased its IL-6 production-enhancing activity. Activity was recovered with re-dimerization of the dissociated GL-RIII.

Tumor necrosis factor alpha (TNF-α). TNF-α is a proinflammatory cytokine with both local and systemic affects. It has the broadest spectrum of pleiotropic activities of all of the cytokines, including inflammation, septic shock, lymphoid organogenesis, and germinal center formation. Immunologically, TNF-α from macrophages is probably the most critical [52]. Boron may affect TNF-α production in chicks and humans. For example, TNF-α concentrations were elevated in the culture medium of pelvic cartilage isolated from chick embryos after they were incubated with boron as a 3% boric acid solution [53]. Likewise, TNF-α concentrations were elevated in the culture medium of human fibroblasts after incubation with 0.25% boric acid. There was also an increase in the amount of total cellular mRNA present and the two TNF-α mRNA bands visible after treatment with boric acid were undetectable before treatment [54]. Because the treatments represented elevated, non-physiological amounts of boron, there is a need to determine whether the effects of boron on TNF-α production are of physiological importance.

Diadenosine phosphates (Ap_nA). Ap_nA molecules are present in all cells with active protein synthesis and function as signal nucleotides associated with platelet aggregation and neuronal response. The Ap_nA are putative "alarmones" which reportedly regulate cell proliferation, stress response, and DNA repair [55]. Recent findings indicate that Ap_6A, Ap_5A, Ap_4A, and Ap_3A have higher affinities for boron than any other currently recognized boron ligand present in animal tissues including NAD^+ [56]. At physiologic pH, the adenine moieties of Ap_nA are driven together by hydrophobic forces and stack interfacially [57]. Stacking of the terminal adenine moieties brings their adjacent ribose moieties into close proximity, a phenomenon that apparently potentiates cooperative boron binding between the opposing riboses (Structure 9).

Boron and Serine Proteases

The known ability of boron to form covalent bonds with the nitrogen atom of amine groups (as described above) and the observation that boron binds near the coordination iron site of hemerythrin (the nonheme iron-containing, oxygen

transport protein of the sipunculid worm, *Golfingia gouldii*) [58] suggest the possibility of a large array of biochemicals other than polyols that can react with boron to form complexes. The serine proteases are major proteolytic enzymes (i.e., elastase, chymase, and cathepsin G) released by activated leukocytes that, in addition to degrading structural proteins, have many essential regulatory roles in normal inflammation, including control of the blood fibrinolytic system (e.g., thrombin) and the coagulation system (e.g., coagulation Factor Xa) [59]. The boron atom is thought to inhibit the serine proteases by forming a tetrahedral B adduct (the transition-state analog) that mimics the tetrahedral adduct formed during normal substrate hydrolysis [60]. The adduct includes a covalent bond between boron and a specific nitrogen at the active site of these enzymes (Structure 5). Nanomolar concentrations of certain synthetic peptide boronic acids, including MeO-Suc-Ala-Ala-Pro-acetamido-2-phenylethane boronic acid, effectively inhibit chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase in vitro [61]. Natural, simple unsubstituted boric acid compounds (e.g., sodium borate) also affect serine protease activity. For example, the serine protease, thermitase (E.C. 3.4.21.66), is partially inactivated by hydrogen peroxide in the presence of 50 mM sodium borate [9].

Boron and the Respiratory Burst

NADPH. When neutrophils and other phagocytes are exposed to appropriate stimuli, they begin to produce large quantities of superoxide. During this process, the phagocytes consume much more oxygen than that needed for the generation of metabolic energy required for phagocytosis, a phenomenon termed respiratory burst. The primary electron donor for the reduction of oxygen during respiratory burst is NADPH [62] and the source of the NADPH for the respiratory burst comes mainly from the reduction of $NADP^+$ in the pentose-phosphate pathway. As described above, this pathway is regulated by boron in plants by inhibiting the activity of two critical enzymes in the pathway [13].

Reactive oxygen species (ROS). When neutrophils invade inflamed areas of the body, they release, among other substances, ROS, including the hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2). If not properly controlled, ROS cause severe damage to healthy tissue and lead to a myriad of inflammatory diseases. Superoxide dismutase (SOD) is an oxidoreductase that serves to dismutate superoxide anions. Boron may be important in the oxidant scavenging process because boron supplementation increased erythrocyte SOD concentrations in men and postmenopausal women [63]. SOD concentrations increase during increased oxidative metabolism or in response to noxious stimuli. It remains to be determined whether SOD concentrations increased because boron may have induced free radical formation (unlikely) or whether boron improved antioxidative capacity. Glutathione peroxidase reduces hydrogen peroxide by means of reduced glutathione and the intracellular reduction of glutathione requires NADPH and glutathione reductase [64].

γ-glutamyl transpeptidase. Boron may exert its influence on the oxidant scavenging process through direct action on γ-glutamyl transpeptidase (GGT).

That enzyme is the major catabolic enzyme for glutathione and its derivatives. Serine-borate complex is a transition-state inhibitor of GGT [65]. By that mechanism, serine-borate apparently elevated the concentrations of GSH in cultured fibroblasts taken from individuals suffering from glutathione synthase deficiency [66]. Thus, the available evidence is consistent with the hypothesis that boron protects against oxidative damage by an unknown mechanism.

Boron and the Immune Response

There are several lines of evidence that dietary boron exerts influence on immune function in humans and animal models as described below. The mechanisms for these systemic effects have not yet been identified but could very easily involve one or more of the biomolecules or processes described above (Fc receptor expression, IL-6, TNF-α, and Ap_nA concentrations and inhibition of the respiratory burst) that affect pain and fever, lymphocyte activation, and natural killer cell concentrations.

Pain and fever. The Unani traditional medical system in India uses sodium tetra-borate or borax as an ingredient of some prescriptions for treatment of inflammatory diseases including joint pain [67]. In the only reported controlled human study for examination of dietary boron and pain [68], twenty patients presenting radiographically confirmed osteoarthritis received either daily 6 mg (0.55 mmol) of boron as oral supplements or a placebo for 8 weeks in a double-blind trial. The arthritic individuals who received boron supplements self-reported substantial improvement in subjective measures of their arthritic condition (joint swelling, restricted movement, fewer analgesics for pain relief). In a separate study with rats, boron (10 mg/kg body weight; single dose) as common borax was reported to have anti-arthritic and anti-pyretic activities because it reduced paw volume and fever in albino rats with formaldehyde-induced arthritis in [67].

Lymphocyte activation. The addition of boron in vitro over a range between 0 and 20 μg (1.85 μmol)/mL inhibited proliferation of splenic cells isolated from boron-deprived rats and subsequently stimulated by 0, 5, or 50 μg phytohemagglutinin/mL [69]. Also, physiologic amounts of boron [3 μg (0.28 μmol)/g] added to a boron-low diet (0.2 μg [0.02 μmol]/g) more than doubled serum total antibody concentrations to injected antigen (human typhoid vaccine) in rats [70]. Gilts fed a boron-low or boron-supplemented (5 mg B/kg diet as sodium borate) diet throughout the nursery, growing, and finishing phases exhibited differences in immune function [71]. For example, on experimental d 95, the skinfold thickness response after an intradermal injection of phytohemagglutinin was significantly lower in the gilts that received supplemental boron. Boron did not affect the blastogenic response of isolated lymphocytes to mitogen stimulation or the humoral immune response against a sheep red blood cell suspension. Healthy peri-menopausal women excreted 1.1 and 3.0 mg boron/d during the placebo and boron supplementation periods respectively and exhibited an increased percentage of polymorphonuclear leukocytes during the boron supplementation period [72]. A recent preliminary report [69] suggested that ample (but probably not pharmacologic) amounts of dietary boron (20 μg [1.85 μmol]/g) compared with very low amounts (< 0.2 μg (0.02 μmol]/g), significantly delayed the onset of

adjuvant-induced arthritis in rats (incidence of arthritis at 12 days postinjection with *M. tuberculosis*: <0.2 μg B/g, 41%; 20 μg B/g, 0%).

Natural killer (NK) cells. Circulating NK cells, compared with other lymphocytes, divide rapidly and comprise about 15% of blood lymphocytes [73]. They are considered an important first line of defense against microbial infections because their effector functions of cytolysis and cytokine (TNF-α, IFN-γ, and GM-CSF) secretion are not antigen specific and are activated immediately after contact with infected target cells [74]. Their response to adjuvant (*M. butyricum*)-induced arthritis was examined in rats deprived or supplemented with dietary boron [75]. All rats grew at a normal rate and exhibited signs of inflammation after injection. Dietary boron had an immunomodulatory effect by affecting the circulating concentrations of NK cells. For example, on day 13 after injection, rats fed the boron supplemented diet exhibited higher circulating concentrations of NK compared to those fed the boron low diet. It seems reasonable to conclude that the effect of boron on arthritis in the study was not pharmacological in nature because the total amount of boron in the boron-supplemented diet was only 2 mg B/kg. For purposes of comparison, the concentration of boron in commercial rodent chow, with unknown boron bioavailability, is typically $\sim$13.0 μg/g [76].

Predictions

Based on the structure of known boron-containing biomolecules, it is predicted that several similar biomolecules waiting discovery are signaling molecules that interact with the cell surface and are probably comprised of two mirror or near-mirror halves stabilized by a single boron atom to form a large circular (a macrocyclic) biomolecule.

SUMMARY

Boron is an essential trace element for plants and is beneficial or established as essential for four animal models of human nutrition. In humans, boron appears to be beneficial and under homeostatic control. This review summarized boron chemistry and biochemistry and known essential functions across the biological spectrum as they relate to better understanding of the apparent role of dietary boron in immune function.

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